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# Studies on Flax Retting.

By

T. Tadokoro, *Nōgakushi.*

## Introduction.

The cultivation of flax in Japan is chiefly confined to Hokkaido and its fiber manufacture is performed solely by a joint-stock company, Teikoku Seima Kabushiki Kaisha at Sapporo.

The quality of the product depends largely upon the proper management of the retting process, though other mechanical treatments bear an important relation to it. The retting method followed in Japan at present is a cold water process. An opportunity was given us by the said company to examine this management thoroughly and the necessary materials were placed at our disposal for the present study.

Investigations concerning the nature of the changes involved in flax retting are numerous in Europe and America but as yet no study has ever been made here. The conditions and the methods of retting under which the foreign investigations have been made, differ naturally from those in Japan. Besides, the investigations already made seem to us to be of a partial and incomplete nature. All of the reported microscopical investigations are confined to the fiber bundles and their cementing materials. Such is the case also with chemical studies. So far as we can find, no study has been made which covers the whole extent of the retting phenomena or the changes in different stages of retting.

In the present study we have tried to investigate the nature of the changes in different stages of retting and the chemical nature of the constituents of the flax stem. We have also studied the micro-organisms concerned in the retting process but the results are reserved for a future report.

The microscopical studies were made under the direction of Prof. Dr. K. Shibata and the chemical studies under Prof. Dr. K. Oshima, to whom the author wishes here to express his hearty thanks for the valuable suggestions and kind courtesies extended to him.

### I. Materials for the Study.

The material used in our studies was the flax, typical in size, qualities and ripening, and was produced in the vicinity of Sapporo, in the years 1908 and 1909.

The retting was done in a large crate, according to the usual method followed in Hokkaido. The methods practiced commonly here are of two kinds, the one is a large crate system and the other, a small frame system. The former resembles the Loppens and Deswarté system and the latter, the pool retting or courtrai method, adopted in Europe.<sup>1)</sup>

In the large crate system a pit about 60 feet long and 9 feet wide is dug in a location, where the inlet and outflow of water can easily be managed and in it is placed a large crate of wood work. Each crate will hold about 8,000 kin (13,200 lbs.) of flax stems.

In the small frame system, frame works of wood, each having a lath floor and measuring about 12 feet long, 9 feet wide and 3.7 feet deep are floated in a pool which usually measures about 180 feet long, 60 feet wide and 5.5 feet deep. Each frame will hold 1,600 kin (2,600 lbs.) of flax stems.

When the fermentation is complete, the bundles are drawn out and are

1) For the details of the methods see C. R. Dodge—*Flax culture for seed and fiber in Europe and America*—(U. S. Dep't. of Agr. Fiber Investigation Office, Report No. 10, (1899) p. 20.

placed close together, standing on the bank for at least 6 hours to allow the water to drain off. When they become firm enough to be transported, they are spread out for drying. When the drying has attained the proper degree, they are again bound into large bundles and kept in a store house for future technical manipulations.

The duration of immersion depends largely upon the temperature of water and air, the qualities of stems and other natural conditions.

In our study, the retting period ranged from the twelfth to the eighteenth, inclusive, of August 1909. During our observation the temperature of the water in the crate varied as follows:

Table 1.

Date.	6 A. M.	2 P. M.	7 P. M.	
August	Upper Middle Lower part. part. part.	Upper Middle Lower part. part. part.	Upper Middle Lower part. part. part.	Weather
12th.	19°C. 18°C. 18°C.	20°C. 19°C. 19°C.	20°C. 19°C. 19°C.	fair
13th.	18°C. 19°C. 19°C.	24°C. 24°C. 23°C.	24°C. 24°C. 23°C.	„
14th.	20°C. 20°C. 19°C.	22°C. 21°C. 21°C.	22°C. 21°C. 21°C.	„
15th.	20°C. 19°C. 19°C.	23°C. 26°C. 22°C.	23°C. 22°C. 22°C.	„
16th.	23°C. 22°C. 22°C.	24°C. 22°C. 22°C.	23°C. 23°C. 23°C.	„
17th.	21°C. 21°C. 21°C.	23°C. 22°C. 22°C.	25°C. 22°C. 22°C.	„
18th.	21°C. 21°C. 21°C.	22°C. 22°C.		

From the table, we see that the temperature in the upper part of the crate is generally a little higher than that in the middle or lower portion and that it is lowest in the early morning, rises gradually up to the middle of the day and keeps on until seven P. M., showing that the most powerful and active period for organisms concerned in retting must be in the afternoon. From this fact, we notice that the proper time for drawing out the retted stem from the crate should be either in the morning or in the evening. Our samples were selected from the retting tank every 24 hours in the morning

and treated in the usual manner.

Water used in our experiment was supplied from the Sosei canal which passes through the city of Sapporo and contained a relatively large amount of organic matter, with a slightly acid reaction. During the retting the acidity was somewhat increased, as naturally might be expected.

## II. Microscopical Studies.

The samples, collected in different stages of the retting period as mentioned in the previous chapter, were subjected to research as to their histochemical changes during the retting. The result on the retted stem has been compared to that of the original stem.

Microchemical studies on flax stems, reported up to the present time, are quite numerous<sup>1)</sup> but their results agree closely with each other and prove that the bast fiber cells are bound not only with calcium pectate but also

1) Tine Tammes. *Der Flachs Stengel.*

Franz Ritter und Höhnel.—*Mikroskopie der technische verwendeten Faser Stoffe.*

A. Herzog.—*Beiträge zur Kenntnis der Flachsfaser*—*Oesterreiche Chemiker Zeitung*, (1898), No. 10. p. 31.

K. Saito.—*Anatomische Studien über wichtige Faserpflanzen Japans mit besonder Berücksichtigung der Bastzellen*.—*Jour. of College of Science, Imp. Univ. Tokyo*, Vol. XV. (1901) p. 3.

W. Omelianski.—*Über die Trennung der "H" und "CH<sub>4</sub>" von der Cellulose*.—*Centr. bl. f. Bakt. II. Abt. Bd. 11*. p. 369.

W. Omelianski.—*Histologischen und Chemischen veränderungen der Leinstengel unter Einwirkungen der Mikroben der Pectin- und Cellulose-gährungen*.—*Centr. bl. f. Bakt. II Abt. Bd. 12*. p. 33.

J. Behrens:—*Untersuchungen über der Gewinnung der Hanffaser durch natürliche Röstmethoden*.—*Centr. bl. Bakt. II Abt Ed. 12*. p. 161.

J. Behrens.—*Über die Tauröste von Flachs und Hanf*.—*Centr. bl. f. Bakt. II Abt. Bd. 10*. p. 524.

C. van Iterson.—*Die Zersetzung der Cellulose durch aerobe Mikroorganismen*.—*Centr. bl. f. Bkt. II Abt. Bd. IX*. p. 689.

M. W. Beijerinck und A. van Delden.—*Über d. Bakterien, d. bei d. Maceration d. Leinstengel*—*Jahr. der. Fortschrift Agr. chem.* (1905) p. 463.

with woody substances and that the middle lamella of fibers are therefore not destroyed in the retting process, though the bast fibers are separated from the parenchyma which are destroyed by micro-organisms.

In 1904 D. Störmer<sup>1)</sup> made further investigations on this point and ascertained that the portions which gave a wood reaction with phloroglucin and hydrochloric acid or anilin sulphate are middle lamella of the bast fiber cells and xylem portion, and that the bast fibers themselves and cell-walls of cortical parenchyma gave no color reaction. When the middle lamella were treated with ruthenium red, they stained, like parenchyma, intensely red, showing the presence of pectin substance; but, on the retted stem, the middle lamella of fibers showed only the hadromal reaction, indicating the destruction of pectin substance during the retting process.

In our microscopical study we used the section of the stem 5-10  $\mu$  in thickness, prepared either by the celloidin imbedding or the common paraffin imbedding.

For the celloidin imbedding, we must first prepare water free objects. To attain this object, the sample was treated first in dilute alcohol, then in strong alcohol and lastly in absolute alcohol. Then the objects were kept in a mixture of the same volume of alcohol and ether from 6-10 hours and then in the dilute celloidin solution, containing 2 grams of celloidin in 100 c.c. of the mixed solution; then in the [double] strong solution of celloidin from 2-3 days and before the paraffin imbedding, the objects were kept in bergamot oil from 12-24 hours. After the treatment, the objects were imbedded in paraffin according to the usual method.

### I. Anatomical Structure of Flax Stem.

Before we go into the results of our observations upon the changes of structure during retting, let us briefly describe the anatomical structure of the

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1)—K. Störmer.—*Über die Wasserröste des Flaches.*—Centr. bl. f. Agr. chem. (1905) p. 841.  
Centr. bl. f. Bakt. II Abt. Bd. f. (1904) p. 351.

mature flax stem. The stems used for the observation of anatomical structure were those grown on the field, rather thickly sown, according to the usual method of cultivation in Hokkaido.

1. The epidermis is a layer with many stomata; its cells are always elliptical. The outer wall is almost even and close to the cuticle and more resistible than the inner wall. The thickness of the cuticle varies according to the height and diameter of the stem. The cuticle has strong resisting power against the action of organisms and other physical conditions.

2. The outer cell of the cortical parenchyma resembles an epidermal cell in form but the other cells are so slender that we can easily distinguish them from the former.

3. The phloem-parenchyma consists of 1-5 cell layers and the cells between the fiber bundles have more or less irregular form. The fibers have a long, stretched, spindle form, with almost sharp pointed ends. A cross section of the fiber in the middle part of the stem has an angular form with 3-7 sides, but that of the upper or lower part of the stem has a round oval form. The walls are quite thick and the lumen in the lower and the upper part of the stem are large and have many striations and pits.

4. The xylem portion consists of the vessels, trachids, wood-parenchyma and medullary rays. The diameter of all elements become smaller from inner to outer layers.

5. The pith consists of the thin-walled cell layers which enclose the large pith cavity in the center of the stem. In the upper part of the stem, the cavity is very narrow or completely filled. The cells increase in size from outer to inner layer.

## 2. Anatomical Changes during Retting.

Retting was divided into three periods, i. e., the first, the middle and the last and each period covered nearly the same number of days. At the end of the last period, not only the separation of the cambium layer and the isol-

tion of the fiber but also the destruction of the cuticle took place. The following are the chief results of our microscopical observations upon the changes of the stem during retting.

1. The cuticle strongly resists the action of micro-organisms and other physical conditions, since we observe that, at the end of the middle period and in the last period, it is gradually separated with the dissolution of the other cell-walls.
2. The outer walls of the epidermal cells are less destructible than the inner. The inner walls begin to dissolve and allow their contents to exude, at the end of the first period; in the middle of the last period they were destroyed completely with the other parenchyma walls.
3. The cortical parenchyma walls are dissolved almost completely, in the middle of the last period, after the destruction of the cambium layer.
4. The dissolution of the internal cells of the fiber-bundles takes place at the same time as that of the cortical parenchyma, but the isolation of the fiber itself is observed at the middle or the end of the last period.
5. The separation of the cambium layer occurs at the end of the middle period. It begins to separate from the xylem portion and dissolves gradually so that the separation of fiber-bundles from the woody portion was found to reach completion at the beginning of the last period.
6. The changes of the xylem portion could not be observed even at the end of the last period.

From the above observations, we conclude that the separation of the cambium layer from the xylem portion takes place first and then follows the destruction of the cortical parenchyma and some parts of the epidermis; the isolation of the fiber itself and of the cuticle comes then in order.

### 3. Microchemical Observations.

#### A. Reagents and Coloring Matters.

1. Zinc chloride iodin solution. 20 parts of zinc chloride, 6.5 parts

of potassium iodide and 1.3 parts of iodin are dissolved in 10.5 parts of water. The solution stains the cellulose reddish or bluish violet, the woody portion yellowish or brown and the wall, consisting of pectin substance, yellow.

2. Congo red. It is easily soluble in water and stains the cellulose red.

3. Safranin solution. A saturated safranin solution in alcohol is mixed with the same volume of water. It stains the pectin compounds yellowish red.

4. Ruthenium red. It stains the pectin compounds intensely red.

5. Methylen blue. It stains the pectin compounds violet.

6. Millon's reagent. The equal quantities of mercury and nitric acid are mixed and diluted in the same volume of water. It stains protein brick-red in a short time.

7. Ammonium copper oxide solution. The solution is prepared fresh by pouring the concentrated ammonia over the copper foil or powder and allowing to stand for a day. It dissolves the cellulose easily.

8. Phloroglucin and hydrochloric acid. A few drops of phloroglucin and concentrated hydrochloric acid produces a red color on the cross section of the lignified cell-wall.

9. Fehling's solution. Three solutions are prepared separately, each containing 35 grams of copper sulphate, 173 grams of Seignette salt (potassium sodium tartarate) and 120 grams of caustic soda in a liter of water respectively. Equal volumes of the solutions are mixed and diluted with 2 parts of water just before using. The sections are put into this solution and warmed over a flame until bubbles appear.

10. Sudan III. Sudan III. is a good coloring matter for fat. It stains fat, wax and cutin red.

11. Osmic acid. It stains fat and protein brown.

12. Alcanna tincture. It is prepared after Guigard's method in the following manner. 10 grams of alcanna were extracted with 30 c.c. of absolute alcohol. Evaporate the extract and treat the residue with 5 c.c. of acetic

acid and 50 c. c. of 50% alcohol. The solution stains fatty substances red. It also reacts on wax and cutin.

### B. Results of the Observation.

1. Cellulose. On the cross section of the stem, we applied congo red and zinc chloride iodin solution. The cells and cell layers which are stained with congo red are the cell-walls of the cortical parenchyma, phloem-parenchyma, epidermis and fibers. The middle lamella of the fibers are faintly stained but the fiber themselves are stained more intensely than the other cell-walls.

With the zinc chloride iodin solution, the bluish violet coloration was observed on the same tissues as above.

From the results we may conclude that the cellulose is a constituent not only of the fibers, but also of the cell walls of the cortical parenchyma, phloem-parenchyma and epidermis, though its quantity is very small.

We could not observe any change of cellulose at the end of the first period of the retting but in the middle or in the last period, the destruction of cell-walls of the cortical parenchyma, phloem-parenchyma and epidermis was noticed. On the other hand, the cellulose reaction on the fibers became more intensive, with the progress of the retting period.

2. Pectin compounds. For the study of pectin compounds, two methods were employed. The one was direct examination with ruthenium red, and the other was staining with methylen blue and safranin, after the dissolution of the cellulose with ammonium copper oxide solution. The characteristic red color with ruthenium red was observed on the middle lamella of the fiber on the cell-walls of the cortical parenchyma, cambium and epidermis and also on the middle thickening layer of fiber cells. Among them the middle lamella of fibers were stained intensely red.

With methylen blue, the middle lamella of fibers were stained a deep violet, the inner thickening layer of fibers, the cell-walls of the cortical

parenchyma, phloem-parenchyma, cambium and epidermis were stained light brown to violet.

The yellowish red color with safranin was observed on the cell layers mentioned above.

In the beginning of the last period of the retting, the middle lamella of fibers showed almost no reaction of pectin compounds, but the cell-walls of the cortical parenchyma, phloem-parenchyma, cambium and epidermis still showed a faint but distinct color and in the end of this period, the color was shown only on the inner thickening layer of fibers.

3. Protein. With Millon's reagent, only the fiber lumen was stained brick red, the other cortical parenchyma, cambium and epidermis as well as the xylem portion being stained very faintly brown.

4. Lignin. The red color produced on the use of phloroglucin and hydrochloric acid is a characteristic test for lignin. On the cross section, the reaction was observed only in the xylem portion and showed no change even at the end of the retting.

5. Tannin. When potassium bichromate was applied on the cross section, we observed the reddish brown color in the contents of the epidermal cells and this color almost disappeared at the end of the middle period. This phenomenon must be due to the effusion of the tannin from the cells, accompanying the destruction of the inner walls, as the retting proceeds.

6. Glucose. The quantity of cuprous oxide formed by Fehling's solution on glucose was found to be very little, the greater part of the glucose present being dissolved during the retting.

7. Fat, wax and cutin. For the examination of these ingredients, we used three reagents, i. e., sudan III, osmic acid and alcanna tincture. The cuticle, cortical parenchyma, and fiber lumen gave the characteristic reactions with sudan III. On the other layer we could not observe any reaction. The same results were obtained with two other reagents. From what we observed, we can assume that the cuticle contains cutin, the cortical parenchyma, some minute fatty globules, the pith, many fatty or wax-like globules and the fiber lumen, fatty substances besides protein.

Of these substances, we observed further that some of the cutin which is

separated mechanically with the destruction of other cell layers, as well as the fatty substances, are lost in the last period of the retting.

We summarize the principal results of our observations as follows.

1. The greater part of cellulose remains unchanged after retting but the small quantity which forms the cell-walls of cortical parenchyma, cambium, phloem-parenchyma and epidermis is lost in the end of the last period.
2. Pectin compounds which constitute the middle lamella of the fibers and the cell-walls of cortical parenchyma, cambium and epidermis are dissolved almost completely at the end of the retting.
3. The quantity of protein is very small, so its loss is almost insignificant.
4. Tannin found in the contents of epidermal cells, diminishes gradually in the process of retting and in the middle or in the last period it is dissolved out completely.
5. Lignin which forms the cell-walls of the xylem portion remains nearly unchanged.
6. The small quantity of glucose present in the rest of the cell-sap is largely dissolved out and only a trace is found in the xylem portion, after the retting.
7. The quantity of the fatty substance lost during retting is very small, but a part of the cutin is separated at the end of the last period.

### III. Chemical Studies.

In order to obtain a definite idea as to the amount and nature of substances lost or changed during retting, we have made the following chemical experiments. Along with these studies it seemed to us to be of practical use as well as of much scientific interest to investigate the chemical nature of gummy substances in the flax stem, since it has been shown that they are the principal matters which suffer change during retting.

### 1. The Loss of Weight.

The loss of weight in the stem during retting depends upon the retting methods and the retting grade which is again governed by the temperature and change of water.

In Europe<sup>1)</sup> the loss of weight is calculated as about 30% of the original weight in dew retting, about 25% in water retting and 40-50% in mud retting.

In United States,<sup>2)</sup> it is estimated at about 18-18.5% in common water retting, while in double retting about 14.5-15.5% in the first watering and about 8.8-9.0% in the second watering.

In Hokkaido, it is usually assumed to be about 19-20% of the original stem.

Results of our experiments on this point are shown in the following table.

Table 2.

Expt.	Total loss.		Real loss. in % of dry matter.	Difference. in % of dry matter.
	in % of air dry stem.	in % of dry matter.		
I. ....	18.56	19.15	14.95	4.2
II. ....	17.51	18.79	14.89	3.9

On the first experiment, we used air dry stem, produced in Hokkaido in the year of 1908 and 1909 and retted in canal water at 16°-21° C. for 9 days, changing the water twice, and on the second experiment for 8 days. For the comparison, we determined what we call here "real loss" which is the loss of weight suffered by the stem proper, or the stem free from the roots, terminal ends and remaining leaves. It is to be noticed that in common retting, the

1) R. Kuhnert. *Der Flachs, seine Kultur u. Verarbeitung*, pp. 87-119.

2) Charles Richards Dodge. *A report on flax culture for seed and fiber in Europe and America*, pp. 68-72.

roots, the terminal ends etc. are not removed from the stem. From the results we see that the real loss is always much smaller than the total loss, the difference being about 4%. This must be due to the separation of remaining leaves, seeds, seed capsules and soil particles which are attached on the roots and terminal ends.

That a greater part of the loss takes place already at the end of first period of the retting, is proved by the results of our experiments shown in the following table.

Table 3.

Retting period.	Total loss.		Real loss.	
	in % of dry matter.	Ratio.	in % of dry matter.	Ratio.
End of the first period...	14.00	73	8.21	55
End of the middle period.	15.70	82	11.53	77
End of the last period...	19.15	100	14.95	100

The change of water increases the loss of weight in the stem during retting, as the following results of our experiments show.

Table 4.

Expt.	Water changed.	Water unchanged.	Difference.
I .....	18.56 %	14.7 %	3.86 %
II .....	17.51 %	13.6 %	3.91 %

From the table we see that by changing the water twice during retting the increase of the loss of about 4% is secured.

On the other hand, the extension of the period over the usual time has but slight effect upon the loss of weight. In our experiments when the period was made twice as long as usual without changing the water, the loss of weight was found to be 16.8% of air dry stem, vis. 14.7% in the usual period.

The loss of weight suffered by the stem during retting is caused by the combined action of micro-organisms and the extractive power of water.

In order to get an idea of how large a part each of these factors plays in causing the loss, we made the following experiment.

A definite quantity of air dry stem is taken into flasks containing a definite quantity of canal water. To one series of flasks are added a few drops of formalin, to prevent the propagation of micro-organisms, while to the other series no formalin is added. Arrangements were made with filter paper or cotton to prevent the aerobic retting. The results of the experiment follow.

Table 5.

Experiment. (16-21° C.)	Loss due to the combined actions.	Loss due to the extractive power of water alone.	Loss due to the extractive power of water in % of that of combined actions.
after 7 days .....	10.2 %	5.7 %	56. %
after 12 days .....	17.2 %	10.2 %	59. %

Generally, the extractive power of water is proportional to the quantity and temperature of water, and the duration of immersion. In experiments cited, the difference in results is caused solely by the difference of duration, as the amount and temperature of water were kept as similar as possible in both series. It is to be noticed that the loss of the stem due to the extractive power of water alone is more than half of that due to the combined actions.

The loss of the dry matter is a measurement of the retting grade and it is important for the fiber manufacture to increase the loss of the stem without injurious effects upon the qualities of fiber and tow production.

If the production of finer tows and fibers are desired, the double retting is to be recommended, since they are more easily obtained from the well retted stem than the incomplete one. The loss which is estimated practically in Hokkaido is smaller than that in Europe, this is probably owing to the incomplete change of water.

From the above experiments, we may conclude as follows.

1. In case the water is changed, the loss of weight in the total stem is about 18-19% but on the basis of the stem proper it amounts to about 15%.

2. In case the water is not changed, the loss of weight in the total stem is about 13-15% or about 4% less than that in the common water retting.
3. The loss of weight due to the extractive power of water is about 55-60% of total loss.
4. The greater part of the loss takes place before the end of first period of retting.

## 2. Composition of Flax Stem and their

### Changes in Retting.

Investigations on the composition of flax and their changes involved in retting are but few. In 1854 Hodges<sup>1)</sup> determined the chief constituents of flax fiber and found 82.5% of cellulose, and 7.6% of sugar, gums and pectin compounds. Omelianski<sup>2)</sup> reported in 1904, results of his investigations concerning the amounts of pectin compounds and cellulose in the retted as well as in the control flax stem. For the study of pectin compounds, he treated 5 grams of the stem with alcohol for half an hour, washed with water and the residue was then extracted with 250 c. c. of 2% hydrochloric acid solution for half an hour. To the extracts was added an equal volume of strong alcohol, to precipitate the pectin compounds. In the extract obtained from the control stem some precipitate of pectin compounds was formed but none in that from retted stem. It is evident from his results that the pectin compounds suffer decomposition in retting process. On the other hand, the amount of cellulose seems to remain unchanged. In his determination of cellulose he took 3 grams of sample and mixed with 500 c.c. of Schweitzer's reagent. After standing for 5 days it was filtered through glass wool filter and precipitated with dilute hydrochloric acid. The precipitate was washed with water, dried and weighed. From the control stem 0.6959 grams of cellulose was obtained, while in the retted stem 0.6979 grams of cellulose was found.

1)—Hodges. Chemical gazette. (Des. 1854).

2)—Omelianski. Centr. bl. f. Bakt. II Abt. Bd. 12. p. 33.

In our experiments we analysed both the control stem (not retted) and the retted stem and obtained the following results:

Table 6.

	Control stem.		Retted stem.			Difference.
	in % of air dry matter.	in % of dry matter.	in % of air dry matter.	in % of dry matter.	in % of dry matter of control stem.	
Moisture.....	12.89	—	13.89	—	—	—
Ash. ....	2.31	2.65	0.65	0.75	0.64	2.01
Pectin. ....	2.64	3.03	2.85	3.31	2.82	0.21
Ether extracts. ....	1.11	1.24	1.22	1.42	1.21	0.03
Pentosan-free fiber....	52.18	59.88	52.79	61.31	52.15	7.73
Nitrogen-free extract.	28.9	32.02	28.60	33.21	28.25	3.77

Pentosan-free fiber was determined by König's method 1).

Analysis of other constituents was made by the methods usually followed for plants. From the results it is seen that about 2/3 of the mineral matter in the original stem is lost in the retting process. Of the organic constituents, pentosan-free fiber and nitrogen-free extract suffer most in retting. Both of these groups comprise many chemical individuals. Hence in order to obtain a better and clearer idea about the nature of the substances changed in retting, we determined cellulose, pentosan, gummy substance, pectin compounds, glucose, and tannin in the retted stems, at different periods of retting. The results were calculated also on the basis of the control stem for the sake of better comparison. Cellulose and pectin compounds were determined by Omelianski's method (l. c.). Gummy substance was determined by treating the sample first with 2% ammoniacal water and then the residue was extracted with 5% sodium hydroxide solution and precipitated with hydrochloric acid and alcohol. Pentosan, glucose and tannin were analysed by the usual methods. The results of the analysis are shown in the following table:

1) --Kō in g. --Zs. Unters. Nahrungsmittel., Berlin, 1. (1898) pp. 3-16.

Table 7.

In % of dry matter.		Before retting.	End of first period.	End of middle period.	End of last period.
Cellulose.....	Retted stem.	22.70	24.43	24.43	24.77
	On the basis of control stem.	22.70	22.42	21.53	21.06
	Loss by retting.	—	0.28	1.17	1.64
Pentosan. ....	Retted stem.	22.69	—	—	21.18
	On the basis of control stem.	22.69	—	—	18.02
	Loss by retting.	—	—	—	4.67
Gummy substance. ....	Retted stem.	17.85	—	—	14.94
	On the basis of control stem.	17.85	—	—	12.71
	Loss by retting.	—	—	—	5.14
Pectin compounds. ....	Retted stem.	0.72	—	—	0.42
	On the basis of control stem.	0.72	—	—	0.36
	Loss by retting.	—	—	—	0.36
Glucose. ....	Retted stem.	0.95	—	—	0.0014
	On the basis of control stem.	0.95	—	—	0.0012
	Loss by retting.	—	—	—	0.948
Tannin. ....	Retted stem.	1.79	0.60	0.37	non
	On the basis of control stem.	1.79	0.55	0.33	non
	Loss by retting.	—	1.24	1.46	1.79

From the nature of the methods followed, it should be remembered here that pentosan and gummy substance are identical or at least very much resemble each other in nature and that the pectin compounds, at least in part, must also be the compounds of pentosan. Hence in discussing the results, we take the pentosan only into account, neglecting the other entirely.

From the results obtained we notice the following:

1. Pentosan suffers most in retting process.
2. A small quantity of cellulose is also lost, after the middle period of retting.
3. Pectin is not completely lost. This is apparently in contradiction to the results of Omelianski, but our experience tends to show that Omelianski used too small a quantity of the sample, making his results rather doubtful.
4. Glucose and tannin are completely lost in the retting.

Pentosan-free fiber consists of cutin, cellulose and lignin. Attempt was made to determine the three components separately by König's method, but unfortunately it was found impossible to oxidize lignin with hydrogen peroxide, hence the attempt was given up. Concerning the nature of the changes in pentosan-free fiber we must therefore depend upon the results of microchemical observations.

To know whether the changes above mentioned take place in the bark portion or woody portion of the stem, the following experiments were performed. The stem was carefully divided from the cambium layer into two parts, i. e., bark and woody portions and weighed. The results are as follows:

Table 8.

Stem taken.	Bark portion.		Woody portion.		
	gm.	%	gm.	%	
Control stem.....	16.3276	3.6663	35.5	6.6613	64.5
Retted stem. ....	13.2562	3.7647	28.4	9.4915	71.6

In the retted stem the proportion of the bark portion is far less than in

the control stem, showing that the loss of matter has taken place in the bark portion during retting.

Pentosan and pentosan-free fiber in both portions were determined with the following results.

Table 9.

Bark portion.				
	Pentosan.		Pentosan-free fiber.	
	in % of dry matter.	in % of dry matter of control stem.	in % of dry matter.	in % of dry matter of control stem.
Control stem .....	8.52	8.52	56.40	56.40
Retted stem .....	5.67	4.12	51.00	37.08
Difference. ....	—	4.40	—	19.32

  

Woody portion.				
	Pentosan.		Pentosan-free fiber.	
	in % of dry matter.	in % of dry matter of control stem.	in % of dry matter.	in % of dry matter of control stem.
Control stem.....	29.40	29.40	44.79	44.79
Retted stem.....	31.06	29.39	48.18	43.53
Difference. ....	—	-0.01	—	-0.74

From the table we observe that, in the bark portion, pentosan loses about 50% and pentosan-free fiber about 30% in the retting process, while in the woody portion hardly any loss has taken place.

### 3. On the Chemical Nature of Gummy Substance in Flax Stem.

It has been shown in the previous experiments that the chief constituents of the flax stem changed or lost during the retting is the pentosan or gummy

substance in the bark portion. It seemed to us therefore to be of much scientific interest to investigate the chemical nature of this gummy substance. The finely powdered sample prepared from dry stem was extracted with 2% ammoniacal solution over night. On the following morning, the mixture was filtered off through a "Nutsch" filter with suction, washed with water and the residue was extracted with 5% caustic soda solution for 3 days and filtered again. The filtrate was neutralized with dilute hydrochloric acid and precipitated with alcohol. The precipitate was put on filter, washed with alcohol and ether and dried at 110°C., powdered and preserved for hydrolysis. A part of the precipitate thus prepared was dissolved again in potash solution and reprecipitated with hydrochloric acid. The precipitate thus purified was dried over sulphuric acid in a partial vacuum and used for optical research.

Pentosan and methylpentosan of the substance were determined by the method of Ellet and Tollens<sup>1)</sup> with the following results.

Pentosan in dry matter.	90.1%
Methylpentosan (as Rhamnosan) in dry matter.	11.3%

It is thus seen that the substance at hand consists entirely of pentosan and methylpentosan. To determine the nature of the pentosan, the substance was hydrolyzed and subjected to further investigation.

### Products of Hydrolysis.

#### Method of Hydrolysis.

25 grams of the substance and 250 c. c. of 5% sulphuric acid were put into an Erlenmeyer flask. The flask was provided with a reflux condenser and heated in a boiling water bath for 16 hours. At the end of the stated time, the odor of furfural was appreciable. When cooled it was filtered through a "Nutsch" filter with suction and the filtrate was neutralized with pure

1)—Berlin, Ber. D. chem. Ges., 38 (1905) pp. 492-499.

calcium carbonate and filtered again. The filtrate was concentrated, with the addition of a little calcium carbonate, to about 20 c. c. in a partial vacuum. The solution was extracted, at first with 85% alcohol and then twice with 95% alcohol. The clear solution thus obtained was concentrated to a syrup and preserved for further investigation.

### Qualitative Test.

Qualitative reactions were tested at the outset of the investigation to get a general idea of the chief constituents of the gummy preparation.

1. Microchemical tests were applied to determine the presence of pectin, cellulose and lignin. The presence of pectin was ascertained by staining with congo red or with phloroglucin and hydrochloric acid but none of the characteristic reaction could be observed.

2. A small amount of the substance was heated in a test tube with hydrochloric acid and then a little quantity of phloroglucin and equal volume of concentrated hydrochloric acid were added and heated. The filtrate gave the characteristic absorption spectrum of pentose. On distilling the substance with hydrochloric acid of 1.06 sp. gr., a distillate was obtained which gave the characteristic furfural reaction with anilin acetate. The distillate was also tested for the presence of methyl furfural by the spectrum reaction of Oshima and Tollens<sup>1)</sup>. The presence of simple pentosan as well as methyl pentosan was thus confirmed. The presence of galactan was also examined by oxidizing with nitric acid, but the result was negative.

### Rotatory Power.

The rotatory power of the gummy substance was determined with Schmidt-Haensch half shadow polariscope. The results follow:

a) 0.45 gram of dry matter dissolved in 60 c. c. of 5% caustic potash solution.

$$\alpha D = \frac{1.4 \times 0.346 \times 60}{0.45 \times 1} = -64.6^\circ$$

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<sup>1)</sup>—Berlin, Ber. D. chem. Ges. 34 (1901) pp. 1425-1426.

b) 0.1895 gram of dry matter dissolved in 40 c. c. of 1% caustic potash solution.

$$\alpha D = \frac{0.9 \times 0.346 \times 40}{0.1875 \times 1} = -66.4^\circ$$

#### Detection of Xylose.

The syrup gave the following reactions:

- 1). It reduced Fehling's solution very strongly.
- 2). It rotated the plane of polarization toward the right.
- 3). It gave the characteristic absorption-spectrum of pentose with phloroglucin and hydrochloric acid.
- 4). It produced no mucic acid upon oxidation with nitric acid.
- 5). It gave no ketose reaction with resorcin and hydrochloric acid.
- 6). 2 drops of the syrup were placed on an object glass and were seeded respectively with a crystal of xylose and of arabinose. After 48 hours the drop seeded with xylose showed the formation of many new crystals, while the other remained unchanged.

From the above reactions it is highly probable that the syrup contained xylose.

#### Isolation of Xylose.

The syrup was left untouched about one week, when it was found thickly laden with fine crystals. A little amount of 85% alcohol was added to the syrup, well mixed, filtered with suction and washed with absolute alcohol and ether. The sugar thus obtained was 2.5 grams in weight and slightly yellowish in color, but upon recrystallization from alcohol with the use of animal charcoal, it became perfectly white and left no ash on ignition.

1.13 grams of the carefully dried sugar was dissolved in water and made up into 25 c. c. and polarized in 200 mm. tube, in the half shadow polariscope. Strong birotation was observed.

After 24 hours, the rotation was found to be  $4.75^\circ$  on the scale toward the right. The specific rotatory power is

$$\alpha D = \frac{4.75 \times 0.346 \times 25}{1.13 \times 2} = 18.2^\circ$$

The sugar gave a phenylosazone, melting at 154–155°C. and easily soluble in cold alcohol but not in water. The melting point of the sugar was found to be 143–144°C. The sugar under question is therefore xylose.

#### Detection of Arabinose.

The mother liquor filtered off from the crystals of xylose was allowed to evaporate slowly by itself. It did not show any sign of forming new crystals after standing for a long time. The syrup gave a pentose reaction. Attempt was then made to separate and detect arabinose by means of benzylphenylhydrazin. The latter forms easily with arabinose a hydrazone, which is hardly soluble in 75% alcohol, while xylose-hydrazone is easily soluble in the same medium.

3 grams of the syrup were dissolved in 9 grams of 75% alcohol, to which a solution of 2 grams of benzylphenylhydrazin in 4 grams of absolute alcohol was added and the mixture well shaken. After 3 hours, crystalline precipitates were formed. They were separated by filtration with suction, washed with a small amount of 75% alcohol and finally recrystallized from 95% alcohol. The light yellowish white product was dried over sulphuric acid in vacuum. The melting point was found to be 169.–169.5°C.

0.101 grams of the substance was dissolved in 25 c. c. of water and levo-rotation of 0.3 on the scale was observed.

The specific rotatory power is

$$\alpha_D = \frac{0.3 \times 0.346 \times 25}{0.101 \times 1} = -12.7^\circ$$

The observed specific rotatory power coincides with that of arabinose-benzylphenylhydrazone. The small quantity of the hydrazone at hand did not allow the isolation of arabinose.

From the results obtained we may conclude as follows :

1. The gummy substance in the flax stem consists largely of simple pentosan, with a small amount of methyl pentosan.
2. From the products of hydrolysis of the gummy substance, both xylose and arabinose were identified.
3. The pentosan of the gummy substance is, therefore, made up of both

xylan, and araban, the former apparently predominating in amount over the latter.

#### 4. Summary.

The results of chemical investigations will here be summarized.

1. The loss of the stem in water retting as practiced commonly in Hokkaido is about 18—19% but when the retting water is not changed, the loss will be 14—15%. The loss in the stem proper is about 4% less than the amount above stated. About 50—60% of the total loss is induced by the extractive power of the retting water which is naturally influenced by the frequency of change.
2. Nearly all of the constituents of the stem suffer change in the retting process but those which are most affected are pentosan (gummy substance) and fiber in the bark portion.
3. The gummy substance in the bark portion of the stem is made up largely of xylan and araban, with a small quantity of methyl pentosan.

#### IV. General Conclusion.

The principal results of our investigation hitherto described are here summarized.

1. Cutin is the essential constituent of cuticle, and tannin is found in the epidermal cell. Fiber consists of cellulose with a small quantity of pectin compounds, protein and fat-like substance as its integral part. On the other hand, the cell-wall of cambium, epidermis and parenchyma are made up principally of pectin compounds with a small quantity of cellulose. The middle lamella of fibers is composed mainly of pectin compounds. Lignin forms the chief constituent of the cell-wall of xylem, while pectin compound forms that of the pith.

2. Water retting involves anatomical as well as chemical changes of

flax stem. On the first stage of retting, we observe the destruction of the cambium layer, and then of parenchyma, accompanied with the separation of fiber bundles. As the retting proceeds, the isolation of the fiber itself and the detachment of cuticles take place. Nearly all of the constituents of stems are subjected to chemical changes which are induced by the combined action of micro-organisms and of extractive power of water. The loss of weight in the stem may therefore be taken as a measurement of retting grade.

3. The anatomical and chemical changes take place not on all parts of stem but only on the bark portion i. e., outer layers of cambium. The xylem and pith remain almost unchanged.

4. The essential matters which are lost during retting are pentosan, or gummy substance, in the bark portion and fiber (cutin, lignin and cellulose). Of the three ingredients composing pentosan-free fiber, the lignin remains almost unchanged, cellulose loses its small quantity, accompanying the destruction of surrounding tissues of fiber bundles in the bark portion and cutin is detached mechanically in the later stage of retting, with the destruction of other tissues.

5. Among other ingredients, tannin is lost completely. The larger part of the mineral matters and of glucose are also lost. The quantity of protein and fat are very small and their loss may be neglected in consideration.

6. The gummy substance, or pentosan, in the bark portion of the stem is made up largely of xylan and araban, with a small quantity of methyl pentosan.



# Ueber die Enzymatischen Wirkungen der Frischen Nahrungs- und Genussmittel.

Von

**T. Tadokoro, Nōgakushi.**

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In Japan, wie in jedem anderen Lande, kommen die mannigfaltigsten Arten frischer Nahrungs- und Genussmittel, sowohl pflanzlichen als auch tierischen Ursprungs auf den Tisch. Es kommt ihnen ausser dem Nährwert, den sie besitzen, durch ihre enzymatischen Wirkungen auf die Verdauung noch eine besondere Bedeutung zu.

Das Studium dieser Wirkungen ist für die Kenntnis der Verdauungsvorgänge nicht ohne Wert. Zahlreiche Untersuchungen über die enzymatischen Eigenschaften von pflanzlichen und animalischen Organen und Geweben sind schon vor langem angestellt worden, aber ihr Verhalten zur Verdauung ist noch nicht berührt worden. Wir unternahmen darum die folgenden Versuche zur Bestimmung der enzymatischen Wirkungen und ihrer Kraft. Ueber ihre spezielle Beschaffenheit und Beziehung auf die Verdauung wollen wir in näher Zukunft berichten. An dieser Stelle möchte ich nicht verfehlen, Herrn Prof. Dr. K. Oshima für seine stete liebenswürdige Unterstützung meinen herzlichsten Dank auszudrücken.

### Versuchsmethode.

Der frisch bereitete Presssaft aus den Nahrungs- und Genussmitteln wurde auf folgenden verschiedenen Wegen auf jede enzymatische Wirkung geprüft.

#### 1) Nachweis der Proteasen und Amidasen.

a) Peptase. Für die Ausscheidung von übrig gebliebenen Eiweisskörpern in der Probe, welche der Einwirkung des peptolytischen Ferments ausgesetzt gewesen war, wurde die Kupferhydroxydmethode angewendet.

10-25 ccm der verdünnten Eiereiweisslösung, welche Menge für jeden einzelnen Versuch verwendet wurde, verdünnte man auf 100 ccm, und fügte 1-2 ccm gesättigte Alaunelösung und ein Übermass von Kupferhydroxyd hinzu. Das Gemisch wurde zum Sieden erhitzt, filtriert und 12 Stunden stehen gelassen. Der Niederschlag wurde auf dünnem Filter gesammelt und gewaschen. Für die Bestimmung des Eiweissstickstoffes wurde die Kjehldalsche Methode angewendet.

b) Tryptase. Für die Ausscheidung von übrig gebliebenen Peptonen und Albuniosen in der Lösung, welche der Einwirkung des tryptischen Fermentes ausgesetzt gewesen war, wurde die Fällungsmethode mit Alumenischer Lösung angewendet.

10-25 ccm 2 % iger Wittepeptonlösung, welche für jeden einzelnen Versuch verwendet wurde, verdünnte man auf 100 ccm und fügte 7 ccm Alumenische Lösung hinzu. Das Gemisch wurde filtriert, gewaschen und der Niederschlag für die Stickstoffbestimmung nach Kjehldalischer Methode verwendet.

c) Desamidase. Um die desamidierende Wirkung des Presssaftes auf Glykokoll, Asparagin und Harnstoff zu ermitteln, wurde die Menge des in der Lösung gefundenen Ammons nach der allgemeinen Titrationsmethode bestimmt.

Zu 50 ccm 1 % iger Glykokoll-, Asparagin-, Harnstofflösung, welche

für jeden einzelnen Versuch verwendet wurde, fügte man eine genügende Menge von Magnesiumoxyd hinzu. Das alkaline Gemisch wurde im partiellen Vakuum unter 80° C. destilliert und der befreite Ammonstickstoff in 1/10 Normalschwefelsäurelösung aufgenommen und mit 1/10 Normalnatronlauge titriert.

#### 2) Nachweis der Amylase.

Die diastatische Wirkung vom Presssaft wurde mit der Jodreaktion geprüft und die gebildete Zuckermenge der Lösung, welche der Einwirkung des diastatischen Fermentes ausgesetzt gewesen war, nach der Alliinischen-gravimetrischen Methode bestimmt.

10-15 ccm 2 % iger löslicher Stärkelösung, welche für jeden einzelnen Versuch verwendet wurde, wurden mit Fehlingscher Lösung auf die übliche Art behandelt.

#### 3) Nachweis der Lypase.

Die fettspaltende Wirkung des Presssaftes wurde durch eine Titration mit 1/10 normaler Kalilauge und unter Zusatz einer genügenden Menge von Alkohol bestimmt.

50 ccm 3 % iger Ricinusoleumulsion in 10 % iger „Gummi arabicum“ Lösung, welche für jeden einzelnen Versuch verwendet wurde, wurden mit 1/10 normaler Kalilauge unter Zusatz von Phenolphthalein titriert.

#### 4) Nachweis der Glykosidasen.

Um die glykosidespaltende Wirkung des Presssaftes auf Amygdalin- und Salicinlösung darzustellen, verfuhren wir wie folgt. 25 ccm 1 % iger Amygdalinlösung, welche für jeden einzelnen Versuch verwendet wurde, wurden nun der Destillation unterworfen; in dem so erhaltenen Destillat wurde Blausäure durch Berlinerblau- und Guajaktinkturkupfersulfatreaktion und ausser dem durch den Geruch von Benzaldehyde nachgewiesen. Ein anderer Versuch bestand darin, dass 25 ccm derselben Lösung zur Feststellung der Zuckerbildung aus Amygdalin nach der üblichen Alliinischen gravimetrischen Methode behandelt wurde.

25 ccm 1 % iger Salicinlösung, welche für jeden einzelnen Versuch verwendet wurde, wurden zum Sieden erhitzt, tropfenweise mit verdünnter Essigsäure versetzt und filtriert. Das Filtrat wurde nach dem Erkalten aus-

geäthert, der ätherische Auszug verdunstet, der Rückstand mit wenig Wasser aufgenommen und mit einem Tropfen Eisenchloridlösung versetzt; es trat sofort eine Blaufärbung ein; andere 20 ccm derselben Lösung wurden zur Feststellung der Zuckerbildung aus Salicin nach der üblichen Alliannischen Methode, wie oben gesagt verwendet.

5) Nachweis der Oxidasen.

Die Oxidasewirkung des frischen Presssaftes wurde nach erfolgten Reaktionen erwiesen.

Die Blaufärbung mit Guajaktinktur.

- 2) Die rote, in schwarz übergehende Färbung mit Phenol nach Bouquelot.
- 3) Die violette Färbung mit  $\alpha$ -Naphtol nach Bouquelot.
- 4) Die rote Färbung mit Hydrochinon nach Bertrand.
- 5) Die rote Färbung mit Hippursäure.

6) Nachweis der Katalase.

Die wasserstoffsuperoxyd-spaltende Wirkung des frischen Presssaftes wurde nach volumetrischer Bestimmung des ausgeschiedenen Sauerstoffes nachgewiesen und die Menge des übrigen Sauerstoffsuperoxyds mit Kalipermanganat titriert.

Ein 100 ccm haltender Messkolben wurde mit dem Gemisch des Wasserstoffsuperoxyds und Presssaftes gefüllt und das so befreite Sauerstoffgas in einem Eudiometer gesammelt. Nach dem keine Wirkung mehr zu beobachten war, wurde die Mischung durch 5 ccm konzentrierte Schwefelsäure zerstört und dann die gebliebene Wasserstoffsuperoxydmenge mit 1/100 normaler Kalipermanganatlösung titriert.

Mitteilung I.

Ueber die enzymatischen Wirkungen einiger frischen  
Gemüsearten.

Die . roben wurden aus Udoschösslingen (*Aralia cordata*), Yamswurzeln

(*Dioscorea Batatas*), Kohlblättern (*Brassica japonica*), Salat (*Lactuca sativa*), Gurken (*Cucumis sativus*), Zwiebeln (*Allium cepa*), Ingwer (*Zingiber officinale*), Rettigwurzeln (*Raphanus sativus*) gewonnen.

Jede frische Probe, welche in einem Mörser zerrieben wurde, wurde in einer Handpresse ausgedrückt. Der erhaltene Presssaft wurde auf einen Filter gebracht. Das Filtrat goss man wieder auf den Filter zurück bis es keine Trübung mehr zeigte.

#### Versuch 1. Peptase.

Probe a. 50 ccm gekochte Eiereiweisslösung, welche mit demselben Volumen Wasser verdünnt worden war, wurden mit 25 ccm frischen Presssaft gemischt und unter Zusatz von 5 ccm Toluol im Brutschrank bei 35° C. digestiert.

Probe b. 50 ccm derselben Eiereiweisslösung wurden mit 25 ccm gekochtem Presssaft gemischt und digestiert wie oben.

Probe c. 50 ccm Wasser wurden mit 25 ccm frischem Presssaft gemischt und digestiert.

Nach mehreren Stunden wurde die gebliebene Eiweissmenge von 25 ccm der gemischten Lösung durch Fällung mit Kupferhydroxyd bestimmt, und es wurden die folgenden Resultate erhalten.

Nummer der Proben.	Dauer d. Digestion. Stunden.	Eiweiss-Stickstoff Menge mg.	Eiweissmenge. mg.	Unterschied.
Udo	a. ....	82	90.52	1.02
	b. ....	"	90.98	
	c. ....	"	1.11	
Yams	a. ....	"	82.71	1.85
	b. ....	"	82.85	
	c. ....	"	11.02	

Kohl	a. ....	„	81.62	510.12	
„	b. ....	„	81.50	509.37	0.75
„	c. ....	„	1.83	11.46	
Salat	a. ....	80	54.58	341.12	
„	b. ....	„	54.22	338.87	2.02
„	c. ....	„	2.57	16.06	
Gurken	a. ....	82	43.19	269.94	
„	b. ....	„	43.20	270.00	0.06
„	c. ....	„	1.84	11.47	
Zwiebel	a. ....	54	13.45	84.06	
„	b. ....	„	11.74	73.38	10.68
„	c. ....	„	1.82	11.37	
Ingwer	a. ....	56	17.18	107.37	
„	b. ....	„	16.13	100.81	6.56
„	c. ....	„	2.01	12.56	
Rettig	a. ....	48	21.62	134.82	
„	b. ....	„	21.05	133.98	0.16
„	c. ....	„	4.02	24.02	

Die Versuchsergebnisse zeigen, dass nach mehreren Stunden die von den obigen sieben Proben nur fünf bei 35° C. digestiert wurden, dass jedoch keine merkliche Verschiedenheit zwischen Original- und Kontrollprobe gefunden wurde; so ist es klar, dass der Presssaft der Udoschösslinge, der Yams- und Rettigwurzeln, der Kohl- und Salatblätter keine peptolytische Wirkung hat. Nur die zwei Proben von Zwiebeln und Ingwer zeigen eine schwache Wirkung.

### Versuch 2. Tryptase.

Probe a. 50 cem 2 % iger gekochter Wittepeptonlösung wurden mit 25 cem frischem Presssaft durchgemischt und unter Zusatz von 5 cem Toluol im Brutschrank bei 35° C. digestiert.

Probe b. 50 cem derselben Peptonlösung wurden mit 25 cem gekochtem Presssaft gemischt und unter Zusatz von Toluol digestiert.

Probe c. 50 ccm gekochtes Wasser wurden mit 25 ccm Originalpresssaft gemischt und digestiert.

Nach mehreren Stunden wurde die Peptonmenge von 25 ccm der gemischten Lösungen, welche durch die Tannin Methode gefällt worden waren, bestimmt und die folgenden Resultate erhalten.

Nummer der Proben.		Stunden.	Eiweiss-stickstoff Menge. mg.	Berechnete Peptonmenge. mg.	Unterschied.
Udo	a. ....	82	15.62	39.98	27.65
	b. ....	"	20.22	51.76	
	c. ....	"	9.19	23.53	
Kohl	a. ....	82	9.19	23.53	27.65
	b. ....	"	27.57	70.57	
	c. ....	"	0.91	2.33	
Salat	a. ....	80	8.27	21.17	7.07
	b. ....	"	11.03	28.24	
	c. ....	"	0.91	2.33	
Gurken	a. ....	82	11.03	28.24	23.54
	b. ....	"	20.23	51.78	
	c. ....	"	0.91	2.33	
Zwiebel	a. ....	54	28.49	72.91	0.38
	b. ....	"	28.63	73.29	
	c. ....	"	0.87	2.23	
Ingwer	a. ....	56	25.05	64.13	13.18
	b. ....	"	30.20	77.31	
	c. ....	"	0.65	1.66	
Rettig	a. ....	48	8.09	20.71	0.23
	b. ....	"	8.00	20.48	
	c. ....	"	0.52	1.33	

Die Versuchsergebnisse zeigen deutlich, dass bei der Digestion des Peptons durch den Presssaft aus Udoschösslingen, Kohl- und Salatblättern, Gurken und Ingwerwurzeln, dasselbe mit Sicherheit in die niedrigen stickstoffhaltigen Verbindungen zerlegt wird. Bei der Zwiebeln und der Rettigwurzeln hingegen wurde keine Wirkung nachgewiesen.

## Versuch 3. Desamidasen.

Probe a. Je 50 ccm von 1 % iger Glykokoll-, Asparagin- und Harnstofflösung wurden mit 25 ccm frischem Presssaft durchgemischt und unter Zusatz von 5 ccm Toluol im Brutschrank bei 35° C. digestiert.

Probe b. Je 50 ccm derselben Lösung wurden mit 25 ccm gekochtem Presssaft durchgemischt und wie oben erwähnt behandelt.

Probe c. 50 ccm Wasser wurden mit 25 ccm frischem Presssaft gemischt und wie oben behandelt.

Nach mehreren Stunden wurde der Ammonstickstoff in den geeigneten Proben bestimmt und folgende Zahlen gefunden.

Nummer der Proben.	Digest. dauer. in Stunden.	Ammon-stickstoffmenge mg.			Berechnete Menge mg.			Unterschied.		
		in Glyk.- Lös.	in Aspar.- Lös.	in Harns.- Lös.	Glyk.	Aspar.	Harns.	Glyk.	Aspar.	Harns.
Udo	a. 82	0.90	0.91	5.52	4.67	8.73	11.79	0.01	0.00	9.84
	„ b. „	0.91	0.91	0.91	4.68	8.73	10.95			
	„ c. „	0.46	0.46	0.46	2.34	4.38	0.98			
Yams	a. 82	0.46	0.46	2.76	2.34	4.37	5.91	0.00	0.00	4.92
	„ b. „	0.46	0.46	0.46	2.34	4.37	0.98			
	„ c. „	0.46	0.46	0.46	2.34	4.37	0.98			
Kohl	a. 82	0.91	0.91	0.91	4.68	8.73	1.95	0.00	0.00	0.00
	„ b. „	0.91	0.91	0.91	4.68	8.73	1.95			
	„ c. „	0.46	0.46	0.46	2.34	4.38	0.98			
Salat	a. 80	0.46	0.46	0.46	2.34	4.37	0.98	0.00	0.00	0.00
	„ b. „	0.46	0.46	0.46	2.34	4.37	0.98			
	„ c. „	0.46	0.46	0.46	2.34	4.37	0.98			
Gurken	a. 82	0.46	0.46	0.89	2.34	4.37	1.88	0.00	0.00	0.90
	„ b. „	0.46	0.46	0.46	2.34	4.37	0.98			
	„ c. „	0.46	0.46	0.46	2.34	4.37	0.98			
Zwiebel	a. 54	0.46	0.46	0.46	2.34	4.37	0.98	0.00	0.00	0.00
	„ b. „	0.46	0.46	0.46	2.34	4.37	0.98			
	„ c. „	0.46	0.46	0.46	2.34	4.37	0.98			
Ingwer	a. 56	0.14	0.14	1.29	0.72	1.33	2.71	0.00	0.00	2.43
	„ b. „	0.14	0.14	0.14	0.72	1.33	0.29			
	„ c. „	0.14	0.14	0.14	0.72	1.33	0.29			
Rettig	a. 48	0.46	0.46	0.46	2.34	4.37	0.98	0.00	0.00	0.00
	„ b. „	0.46	0.46	0.46	2.34	4.37	0.98			
	„ c. „	0.46	0.46	0.46	2.34	4.37	0.98			

Bei jeder Probe zeigte sich, dass der frische Presssaft aus den Udoschösslingen, Yamswurzeln, Kohl- und Salatblättern, Zwiebeln, Ingwer und Rettigwurzeln keine Glykokoll- und Asparaginspaltungskraft hat, dass aber der Presssaft aus den Udoschösslingen, Yamswurzeln, Gurken und Ingwerwurzeln den Harnstoff angreift unter Bildung von Ammoniak.

#### Versuch 4. Amylase.

Der frische Presssaft von den oben beschriebenen sieben Gemüsen, ausser der Zwiebel, hat stärkespaltende Wirkung, aber wenn man den Presssaft und die Stärkelösung mischt und digestiert, dann zeigt sich keine Jodjodkalireaktion von Stärke mehr. Wie aus folgendem Versuch ersichtlich ist, hat sich diese Annahme über Erwarten bestätigt.

Probe a. 50 ccm 2 % iger löslicher Stärkelösung wurden mit 25 ccm frischem Presssaft durchgemischt und unter Zusatz von 5 ccm Toluol mit Brutschrank bei 35° C. digestiert.

Probe b. 50 ccm derselben Stärkelösung wurden mit 25 ccm gekochtem Presssaft durchgemischt und mit Zusatz von Toluol wie oben erwähnt behandelt.

Probe c. 50 ccm Wasser wurden mit 25 ccm frischem Saft durchgemischt und der Autodigeston überlassen.

Nach etwa 40-50 Stunden wurde von 10 ccm jeder geeigneten Probe die gebildete Zuckermenge bestimmt.

Nummer der Proben.	Digestionsdauer in Stunden.	Die reduzierte Kupfermenge. mg.	Menge der Glukose. mg.	Unterschied.
Udo	a. ....	42	195.3	25.5
	b. ....	"	148.7	
	c. ....	"	147.0	
Yams	a. ....	40	180.0	46.0
	b. ....	"	88.0	
	c. ....	"	57.6	

Kohl	a. ....	42	230.9	119.9	
"	b. ....	"	151.7	77.5	41.5
"	c. ....	"	142.3	72.3	
Salat	a. ....	42	99.0	50.4	
"	b. ....	"	66.2	33.8	17.6
"	c. ....	"	65.4	33.3	
Gurken	a. ....	40	209.6	107.6	
"	b. ....	"	184.0	94.2	13.4
"	c. ....	"	154.3	78.6	
Zwiebel	a. ....	54	226.9	116.9	
"	b. ....	"	232.0	119.6	0.00
"	c. ....	"	228.1	118.5	
Ingwer	a. ....	56	218.0	112.1	
"	b. ....	"	74.8	38.3	73.8
"	c. ....	"	106.0	54.0	
Rettig	a. ....	48	306.4	159.9	
"	b. ....	"	225.9	116.1	43.8
"	c. ....	"	195.9	100.2	

Die Untersuchungsergebnisse zeigen, dass die sieben Proben, ausser der Zwiebel diastatische Wirkung haben und zwar ist diese am kräftigsten im frischen Presssaft der Ingwerwurzel, nächst dieser kommen die Yams- und Rettigwurzeln und Kohlblätter. Die schwächste Wirkung haben die Udoschösslinge, Salatblätter und Gurken.

### Versuch 5. Lypäse.

Probe a. 50 ccm einer 3 % igen Ricinusoleumulsion, welche mit einer 10 % igen Lösung von „Gummi arabicum“ gemischt worden war, wurden mit 25 ccm frischem Presssaft durchgemischt und mit Zusatz von 5 ccm Toluol im Brutschrank bei 35° C. digestiert.

Probe b. 50 ccm der in Probe a. beschriebenen Ricinusoleumulsion wurden mit 25 ccm gekochtem Presssaft durchgemischt und wie oben erwähnt behandelt.

Probe c. 50 ccm Wasser wurden mit 25 ccm frischem Presssaft durchgemischt und wie oben erwähnt behandelt.

Nach mehreren Stunden wurde die gespaltene Fettsäuremenge mit 1/10 Normalkalilauge unter Zusatz von Phenolphthalein und einer genügenden Menge von Alkohol titriert.

Proben.	Digestions- dauer in Stunden.	N/10 Kalilauge zur Neutralisierung verbraucht. ccm.			
		Probe a.	Probe b.	Unterschied	Probe c.
Udo .....	82	5.1	4.8	0.3	2.1
Yams .....	„	15.6	15.3	0.3	7.5
Kohl .....	„	29.7	7.2	22.5	6.9
Salat .....	80	4.5	4.3	0.2	1.5
Gurken .....	82	3.0	2.9	0.1	1.8
Zwiebel .....	54	9.9	8.7	2.2	4.5
Ingwer .....	56	4.5	4.4	0.1	0.5
Rettig .....	58	1.8	1.4	0.2	0.9

Die lypolytische Wirkung wurde nur in dem Presssaft der Kohlblätter erkannt, bei den anderen zeigte sich kein merkliches Ergebnis.

#### Versuch 6. Glukosidasen.

Probe a. Je 25 ccm 2 % iger Amygdalin- und Salicinlösung wurden mit 15 ccm frischem Presssaft durchgemischt und unter Zusatz von 3 ccm Toluol im Brutschrank bei 35° C. digestiert.

Probe b. Je 25 ccm 2 % iger Amygdalin- und Salicinlösung wurden mit 15 ccm gekochtem Presssaft durchgemischt und wie oben erwähnt behandelt.

Probe c. 25 ccm Wasser wurden mit 15 ccm frischem Presssaft durchgemischt und wie oben erwähnt behandelt.

Nach mehrstündiger Digestion war in stark der Geruch nach Benzaldehyd in den Amygdalinproben. Das Gemisch wurde nun der Destillation unterworfen und das so erhaltene Destillat durch Berlinerblau- und Guajactinkturkupfersulfatreaktion auf Blausäure untersucht. 10 ccm des Gemisches ergaben folgende Mengen:

Nummer der Proben.		Digestions-dauer in Stunden.	Reducierte Cu-Menge mg.	Zucker-menge (als Glukose) mg.	Unterschied.	Blausäure-reaktion.
Udo	a. ....	82	110.6	56.0	0.5	—
	b. ....	„	109.8	55.5		—
	c. ....	„	106.9	54.6		—
Yams	a. ....	82	30.1	16.0	9.4	+
	b. ....	„	11.4	6.0		—
	c. ....	„	3.7	1.9		—
Kohl	a. ....	82	151.3	77.0	9.8	+
	b. ....	„	134.7	68.2		—
	c. ....	„	130.1	66.2		—
Salat	a. ....	80	63.6	32.5	-0.3	—
	b. ....	„	42.8	21.9		—
	c. ....	„	64.1	32.8		—
Gurken	a. ....	82	66.1	33.8	0.5	—
	b. ....	„	65.8	33.3		—
	c. ....	„	64.7	33.0		—
Zwiebel	a. ....	54	107.6	54.5	2.6	—
	b. ....	„	99.6	50.4		—
	c. ....	„	102.5	51.9		—
Ingwer	a. ....	56	70.4	36.0	-3.9	—
	b. ....	„	57.2	29.4		—
	c. ....	„	78.4	39.9		—
Rettig	a. ....	48	298.0	155.4	1.6	—
	b. ....	„	289.2	150.4		—
	c. ....	„	290.0	153.8		—

Die Salicinprobe wurde mit Äther extrahiert; der Ätherauszug in einem Becherglas freiwilliger Verdunstung überlassen und der Rückstand mit Wasser aufgenommen. Die wässerige Lösung wurde auf die Blaufärbung unter Zusatz von einem Tropfen Eisenchlorlösung geprüft. Es wurde somit die

Abspaltung des Saligenins aus Salicin nachgewiesen. 10 ccm des Gemisches wurden der Zuckerbestimmung unterworfen und die nachfolgenden Werte erhalten.

Nummer der Proben.		Digestionsdauer in Stunden.	Reducierte Cu Menge mg.	Zuckermenge (als Glukose) mg.	Unterschied.	Saligeninreaktionen.
Udo	a. ....	82	136.4	69.8	5.8	+
	b. ....	"	124.8	63.5		-
	c. ....	"	121.4	61.6		-
Yams	a. ....	82	11.7	6.9	0.1	-
	b. ....	"	11.0	6.6		-
	c. ....	"	11.9	7.0		-
Kohl	a. ....	82	140.0	71.3	4.1	+
	b. ....	"	131.6	67.2		-
	c. ....	"	130.2	66.2		-
Salat	a. ....	80	67.6	34.3	1.0	-
	b. ....	"	47.9	24.4		-
	c. ....	"	65.2	33.3		-
Gurken	a. ....	82	60.7	31.1	0.4	-
	b. ....	"	59.1	30.3		-
	c. ....	"	59.5	30.7		-
Zwiebel	a. ....	54	111.3	56.5	1.3	+
	b. ....	"	108.5	55.2		-
	c. ....	"	102.5	52.1		-
Ingwer	a. ....	56	77.4	39.3	0.5	-
	b. ....	"	66.8	34.3		-
	c. ....	"	78.4	39.3		-
Rettig	a. ....	48	280.2	145.5	-0.6	-
	b. ....	"	272.1	141.1		-
	c. ....	"	281.3	146.1		-

Die erwähnten Versuche zeigen, dass die Presssäfte der Yamswurzeln und Kohlblätter amygdalinspaltende und die Udoschösslinge und Kohlblätter salicinspaltende Wirkung haben.

### Versuch 7. Oxydase.

Der frische Presssaft aus dem Gemüse zeigt auf die Oxydase die folgenden Farbenreaktionen.

Troben.	Grün mit Guajaktinktur.	Purpur mit Hippursäure.	Gelb bis grün-violet mit $\alpha$ -Naphtol.	Rot mit Hydrochinon.	Rot bis schwarz mit Phenol.
Udo.....	+	+	-	-	-
Yams .....	++	+ nach 3 Stunden.	-	-	+ nach 3 Stunden.
Kohl .....	++	-	-	-	-
Salat .....	+	-	-	-	-
Gurken .....	++	-	-	-	-
Zwiebel .....	+++	+	++	Tyrosinase vorhanden	+
Ingwer .....	++++	+	++		+
Rettig .....	+	-	-	-	-

Die Ergebnisse zeigen, dass auf jede einzelne Oxydase die Wirkungen der acht Gemüsearten mehr oder weniger verschieden und in der Ingwerwurzel am stärksten sind.

### Versuch 8. Katalase.

Probe a. Je 50 cem des frischen Presssaftes der oben beschriebenen Gemüse wurden mit 5 % iger Wasserstoffsuperoxydlösung gemischt unter Zusatz von Toluol auf 100 cem aufgefüllt und im Brutschrank bei 35° C. digeriert.

Probe b. Je 50 cem des gekochtes Presssaftes wurden wie oben erwähnt behandelt.

Probe c. Je 50 cem des frischen Presssaftes wurden mit Wasser gemischt und wie oben erwähnt behandelt.

Stündlich wurden die Volumen des befreiten Sauerstoffes auf den Messröhren abgelesen. Nach mehreren Stunden, als die Befreiung des Sauerstoffes beendet war, wurde die Menge des zurückbleibenden Wasserstoffsuperoxyds mit N/10 Kalipermanganatlösung unter Zusatz von Schwefelsäure titriert und die folgenden Werte gefunden.

Proben.	Das Volumen des befreiten Sauerstoffes,											
	nach $\frac{1}{2}$ Stunden. cem.			nach $1\frac{1}{2}$ Stunden. cem.			nach 4 Stunden. cem.			nach 12 Stunden. cem.		
	a.	b.	c.	a.	b.	c.	a.	b.	c.	a.	b.	c.
Udo .....	15.0	0.5	0.4	21.4	0.5	0.5	25.6	0.5	0.5	27.8	0.5	0.5
Yams .....	10.4	0.7	0.3	26.8	0.7	0.3	30.8	0.7	0.3	32.8	0.7	0.3
Kohl .....	10.2	0.4	2.7	18.2	0.4	2.7	19.1	0.4	2.7	20.2	0.4	2.7
Salat .....	14.5	0.4	0.2	22.3	0.4	0.2	22.5	0.4	0.2	23.1	0.4	0.2
Gurken .....	17.2	0.1	0.3	22.4	0.2	0.3	25.9	0.2	0.3	26.8	0.2	0.3
Zwiebel ...	23.1	0.5	0.2	49.3	0.5	0.2	50.1	0.5	0.2	64.2	0.5	0.2
Ingwer.....	45.2	0.3	0.6	61.1	0.3	0.6	72.3	0.3	0.6	79.2	0.3	0.6
Rettig .....	15.0	0.2	0.5	23.5	0.2	0.5	31.2	0.2	0.5	32.0	0.2	0.5

Aus obigen Resultaten ersieht man, dass jede Probe katalytische Wirkungen besitzt, dass aber ihre Kräfte mehr oder weniger verschieden sind.

### Zusammenfassung.

In dem frischen Presssaft von Udoschösslingen, Yamswurzeln, Kohl- und Salatblättern, Zwiebeln, Ingwer- und Rettigwurzeln wurden die folgenden enzymatischen Wirkungen nachgewiesen.

1. Keine merkliche peptolytische Wirkung wurde in den sechs Presssaftproben von Udo, Yams, Kohl, Salat, Gurken und Rettig gefunden. Eine schwache Wirkung wurde nur in den zwei Proben von Zwiebel und Ingwer nachgewiesen.

2. Die tryptische Wirkung wurde in jedem Safte, ausser Zwiebel- und Rettigsaft, mehr oder weniger stark nachgewiesen. In dem Saft von Kohlblättern ist sie am kräftigsten.

3. Die ammoniakbefreiende Wirkung kommt dem Glykokoll und Asparagin in keinem Saft zu. Harnstoffspaltende Wirkung liess sich in dem Saft der Udoschösslinge, Yams- und Ingwerwurzeln erkennen, obgleich ihre Kraft nicht stark ist.

4. Die diastatische Wirkung wurde in jedem Saft, ausser dem der Zwiebel, nachgewiesen. Bei der Ingwerwurzel ist sie am kräftigsten, dann folgen Yams- und Rettigwurzeln und Kohlblätter. In den andern Presssäften ist sie schwach.

5. Die lypolytische Wirkung wurde nur in dem Presssaft der Kohlblätter gefunden, bei den andern zeigte sich kein erkennbares Ergebnis.

6. Den Amygdalin- und Salicinspaltende Kraft besitzt nur der Saft der Yamswurzel und der Kohlblätter.

7. Die oxydierende Wirkung, welche der Presssaft aller Proben aufweist ist mehr oder weniger verschieden. Im Saft der Ingwerwurzel und der Zwiebel ist sie am kräftigsten.

8. Die katalytische Wirkung wurde in jedem Saft nachgewiesen, doch ist ihre Kraft mehr oder weniger verschieden. Die Kraft des Saftes aus Ingwerwurzeln und Zwiebeln ist am stärksten von allen Arten.

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